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Drug Discovery in Academia

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1.1 Introduction

It is estimated that the global pharmaceutical industry invested more than US$ 1.36 trillion in the decade from 2007 to 2017, and predicted annual spending is assumed to totally sum up to 181 billion for the period to 2020 [1]. At the end of 2019, the 10 largest pharmaceutical companies represented a market capitalization of approximately US$ 1.68 trillion [2].

The tremendous advances in science starting in the 1990s stipulated hopes that the discovery of new medicines would soon turn into an engineerable process. The decryption of the human genome provided a plethora of new target opportunities for exploitation, and the availability of large screening collections, efficient miniaturized high-throughput screening technologies, and computer-assisted methods for hit generation suggested that generation of reasonable lead structures should be feasible for many of these targets. Furthermore, cellular models for early prediction of metabolic liabilities and toxicological risks enhanced the optimization of drug-like properties. However, after 30 years, these hopes did not turn into reality; the number of approved drugs remained approximately constant, at least for the period from 1989 to 2013. In 2019, the Food and Drug Administration (FDA) approved 47 new drugs, 9 of which are biologics (Figure 1.1) [3]. It is an interesting observation that despite the trend to focus research on biologics and small-molecule drug business was said to be dead for several years, the fraction of annual new biological drug approvals is still stagnating at about 25 %.

In an article published in 2011, Stevens [4] analyzed the contributions of publicly funded organizations to current approval rates over a period of 40 years. It is remarkable to note that about 9 % of all approvals (143/1541) were enabled or at least facilitated by public funding. If one compares the contributions for new molecular entities, the rate rises to 13.3 % (64 out of 483). For new molecular entities that

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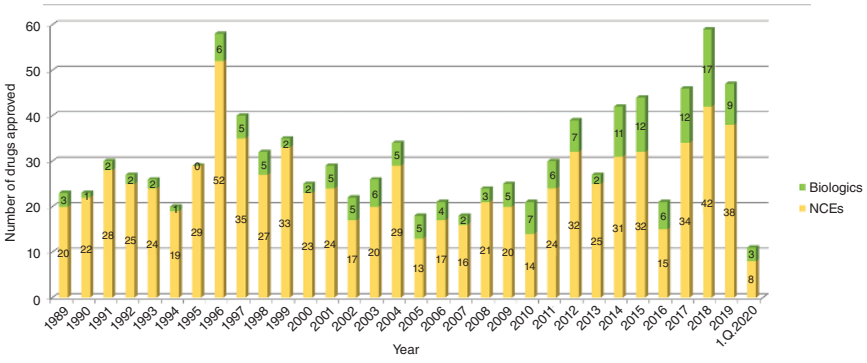


Figure 1.1 FDA drug approvals from 1990 to 2019. Source: Data from Mullard [3].

have been granted priority review, the report cites an impressive 21.1 % (44 out of 209). In a recent study, Nayak et al. [5] confirmed the significance of pharmaceutical research driven by universities and clinical centers. They thoroughly analyzed FDA drug approvals between 2008 and 2019, considering also patent information. Among the 248 approvals of new molecular entities, they identified significant contributions by publicly funded organizations for 62 (25 %) of them. It is puzzling that pharmaceutical ventures with their highly skilled scientists and an infrastructure that is capable of accessing virtually unlimited funds dedicated solely to the purpose of drug discovery did not perform better than these figures tell us. This is even more surprising in light of the fact that provision of new drugs to the pipeline is obviously a vital task in order to maintain the company going in the future and patent lifetime of approved therapies is clearly very limited.

Independent development of a drug to a marketed product is clearly out of scope for any academic. It is estimated that out of pocket costs for approval of a single drug can amount to US$ 1.3 billion, with the majority of this budget being consumed by clinical trials. Also the process needs oversight and management by experienced clinical scientists to optimally set up the studies in order to ensure that a potential beneficial outcome will not be a victim of an underpowered study group or that the selection of the patient population was not optimal.

However, when the clinical trial starts, the selection process of the therapeutic moiety is already completed and the decision on target and approach is taken, from that point on it is the task of the clinicians to see if the generated hypothesis will hold true.

However, academics provide important contributions to drug discovery, using their specific strengths. These can be based on curiosity, expert knowledge in specific areas, exploitation of surprising findings, stimulating follow-up research, and interdisciplinary research resulting from different academic laboratories teaming up, for instance. Different examples of how these specific strengths can lead to successful drug discovery will be discussed throughout this chapter.

1.2 Repurposing Drugs

One contribution ideally suited for academic research is the quest for new indications.

As approved drugs are openly commercially available, researchers, particularly scientists in clinical centers, can – based on patient derived data – generate hypotheses and probe them in a straightforward manner. In this context drug repurposing has attracted a lot of attention as the approach is very straightforward, and the resulting drug has already been demonstrated to be safe, bioavailable, and well tolerated in humans.

Often, this approach is guided by careful observation of disease-accompanying factors and interpretation of the underlying pathology. In particular, changes of symptoms in patients suffering from more than one disease may provide interesting starting points for developing new hypotheses. An example is rituximab, which first was developed for the treatment of cancer. Its discovery will be discussed in more detail during the course of the chapter. Edwards et al. proposed that self-perpetuating B-lymphocytes may play a key role in driving progression of rheumatoid arthritis (RA) and autoimmune diseases [6]. They hypothesized that a CD20 (cluster of differentiation 20) targeted therapeutic, capable of specifically depleting this population of B-cells, may represent an interesting therapeutic option. In 1999, a first case report of a patient suffering from non-Hodgkin's lymphoma in association with inflammatory arthropathy appeared [7]. Within weeks of treatment with a monoclonal anti-CD20 antibody, significant improvement of joint pain was observed, and three months later, the patient was virtually symptom-free and capable of walking distances of 5 miles per day. In a following phase 2 study, positive results of rituximab in patients with RA were demonstrated, [8] followed by further trials. After being able to demonstrate convincing beneficial effects, rituximab was approved for treatment of RA in combination with methotrexate in 2006.

1.2.1 Thalidomide Derivatives

A second example is the utilization of thalidomide, lenalidomide, and pomalidomide for treatment of leprosy and various cancers. After the infamous and tragic history of thalidomide, it would be nearly impossible for any researcher in a big pharmaceutical venture to revive this drug. Being approved in Germany in 1957, thalidomide was frequently used for treatment of morning sickness. As the side effect profile seemed very favorable, it was frequently used by pregnant women. However, in 1961 reports on increased birth defects were reported, which were finally linked to thalidomide. These defects led to a significantly increased mortality at birth as well as to limb deformations, heart problems, and other side effects. It is estimated that more than 10 000 children were born with limb defects. The retraction of the drug from the European market led to introduction of a requirement for more stringent characterization of drug safety during the registration process. Teratogenicity is now one of the flags that will lead to exclusion of a drug from almost any optimization program, as it is difficult to rule out any erroneous use in women of child-bearing

age. However, by 1964, only three years after market withdrawal, Jacob Sheskin from Hadassah University in Jerusalem used thalidomide to treat patents in serious condition of leprosy [9]. In his original publication, Sheskin referred to administering thalidomide to six leprosy patients as a sedative drug; however, to his surprise the disease condition of all six patients improved. The initial study was followed by multiple comparative studies and the clinical benefit, in particular with respect to onset of action, and good tolerability became evident. Thalidomide was finally approved for treatment of leprosy in 1998.

Further research by Judah Folkman's laboratory at Children's Hospital at Harvard Medical School demonstrated that thalidomide effectively inhibited angiogenesis induced by fibroblast growth factor 2, offering a potential mechanistic explanation for the observed limb deformations [10]. Angiogenesis, however, is a hallmark of tumor growth, so in 1997 a trial was started [11] to examine the efficacy of treatment with thalidomide in patients with multiple myeloma, a hematological cancer that was not curable by conventional chemotherapy. A response rate of 32 % was observed. Actually, a first oncology clinical trial of thalidomide had already been performed as early as 1965. Olsen et al. [12] treated 21 patients suffering from various types of advanced cancers with thalidomide. Overall no inhibitory effect of tumor progression was observed in this study. The authors described subjective palliation in one third of patients. Albeit no tumor regression was observed, the authors noted a possible temporary slowing of rapidly progressing cancer in two patients. Interestingly, one of them was suffering from multiple myeloma.

1.2.2 Chemotherapy: Nitrogen Mustards

Another example of drug repurposing is the establishment of chemotherapy for treatment of cancer. Mustard gas was one of the deadliest and most detestable weapons used in World War I, leading to the death of hundreds of thousands of people. Stimulated by findings in medical records of soldiers exposed to mustard gas, which noted that significant changes in the blood composition were observed (notably a pronounced leucopenia), [13] Milton Winternitz, a chemist, teamed up with two pharmacologists at Yale University, Louis Goodman and Alfred Gilman. They decided to investigate potential therapeutic effects of chemical warfare agents for potential treatment of cancer (Figure 1.2). While sulfur lost (S-lost) proved too volatile for therapeutic use, the corresponding nitrogen derivative (N-lost) was more amendable to administration. The hydrochloride salt was significantly safer to handle and solutions for injections could be readily obtained before the anticipated use by dissolution in sterile saline. In a mouse model of lymphosarcoma, rapid tumor regression was observed, albeit the authors noted that required doses were close to toxic levels and tumor reoccurrence was inevitable [14]. However, a first human patient was treated on 27 August 1942, a date that can be regarded as the birth of chemotherapy. J.D. (only the initials of said patient are known today) suffered from advanced non-Hodgkin's lymphoma [15]. He was already treated with radiation therapy, but the tumor still spread and left the patient in a very severe condition. He thus volunteered to participate in an exploratory study, and

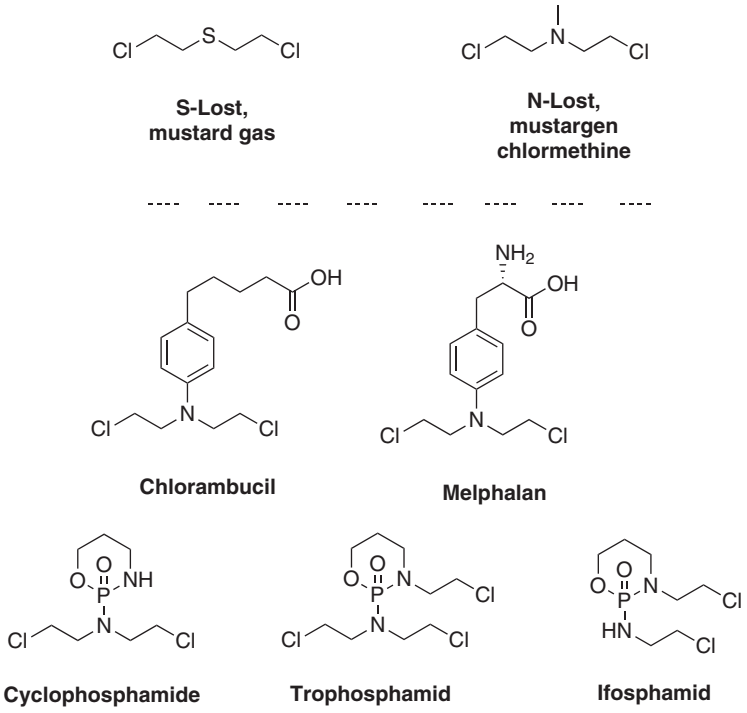


Figure 1.2 S-Lost, N-Lost, modern agents.

indeed daily injections of the drug were able to reverse the symptoms. Rapid tumor regression was observed and his overall condition improved significantly. Unfortunately, the effects were relatively short-lived. A second series of injections was still able to provide some relief from tumor reoccurrence, but a third round of treatment could not improve the patient's condition any more, and J.D. died 96 days after the first injection. However, his lifespan was likely significantly prolonged, and these results spurred further clinical investigation [16]. Overall, beneficial effects have been observed for patients suffering from Hodgkin's disease or lymphosarcoma, albeit the effects were transient and the therapeutic window was narrow. These initial studies had already been performed during World War II, but as chemical warfare agents were the subject of investigation, they were regarded as classified information, which delayed publication until 1946. Publication of these results caused a wave of initial excitement, but the limited duration of treatment effects and the inability to ultimately cure cancer led to a change in mindset and to a widespread pessimism in the medical community. The resulting belief that cancer could be not cured by chemical agents lasted for many years. Still these hallmark results form the foundation of chemotherapy and led to the development of other alkylating agents like chlorambucil, melphalan, and cyclophosphamide (Figure 1.2), which are better tolerated and are still used today in clinical practice. It is noteworthy to correct a historical mistake that is frequently made. The bombing of a ship in Bari during World War II, which led to exposure of the crew to mustard

gas, is often cited as the discovery of mustard's antitumor activity and the discovery of chemotherapy. This is not correct. Despite the fact that severe leucopenia was also observed in affected soldiers, the German air raid on the ships in the harbor of Bari took place on 2 December 1943, more than a year after patient J.D. had been treated. The development of chemotherapy is a fascinating topic, which has been reviewed in appropriate detail elsewhere [17].

1.3 Pregabalin

The discovery of pregabalin by Richard Silverman [18] and coworkers is a great example of successful identification of a small-molecule drug in academia. γ-Aminobutyric acid (GABA) was recognized early on as an important inhibitory neurotransmitter in the brain (Figure 1.3) [19]. The observation that GABA levels and L-glutamic acid decarboxylase (GAD) activity is decreased in a number of pathologies like epilepsy, Alzheimer's, and Parkinson's disease has sparked the search for drugs to increase GABA levels in the brain. Pursued strategies include development of GABA receptor agonists, GABA uptake inhibitors, and inhibitors of 4-aminobutyrate-oxo-glutarate aminotransferase. The latter enzyme is the key catabolic enzyme of GABA. Inhibitory effects of hydroxylamine on γ-aminobutyric acid aminotransferase (GABA-AT) were described already in 1961 [20]. In 1966, inhibition of (GABA-AT) by aminooxyacetic acid was disclosed [21].

It was also demonstrated that inhibitors available at the time demonstrated insufficient selectivity [22]; consequently this approach was rendered as likely to be unsuitable to target epilepsy in humans. Shortly after starting his own laboratory at Northwestern University in Illinois in 1976, Silverman got interested in the biology of GABA-AT and set out to develop chemical inhibitors. He published his first manuscript on the subject as early as 1980 [23]. While his first efforts relied on optimization of irreversible inhibitors, he was not able to overcome the intrinsic non-specificity of these compounds. Specifically, inhibition of L-glutamic acid decarboxylase (GAD) turned out to be an issue. GAD catalyzes the conversion of L-glutamate, an excitatory neurotransmitter to the inhibitory neurotransmitter

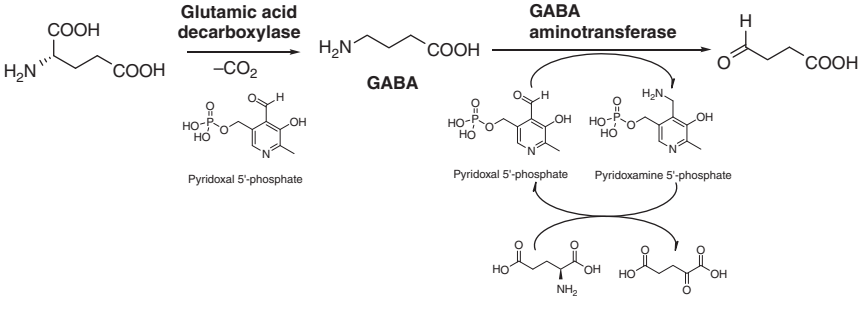


Figure 1.3 GABA biology.

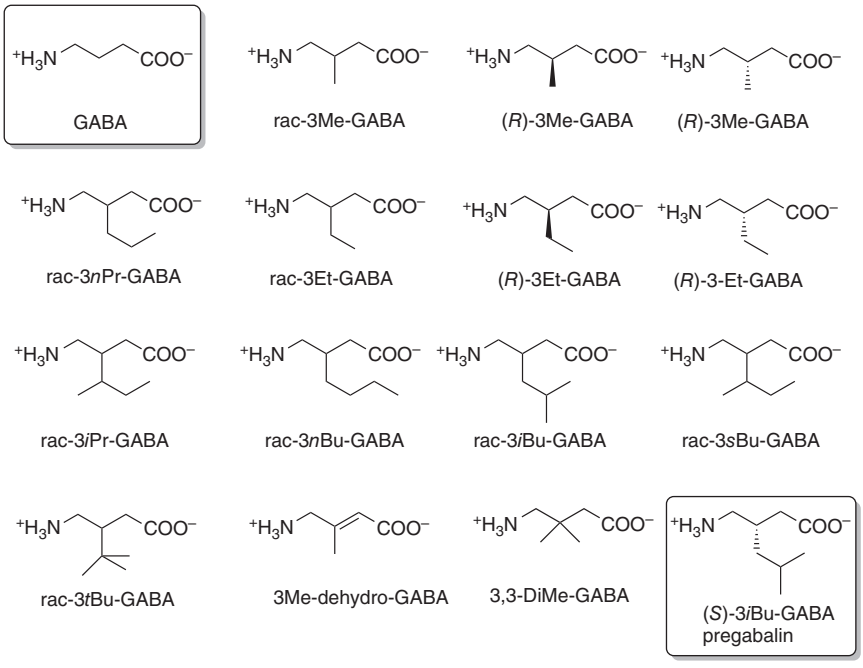


Figure 1.4 3-Me-GABA analogues synthesized by Andruskiewicz and Silverman.

GABA. Inhibition of GAD would consequently lead to a decrease in GABA concentration and thus be highly undesirable.

In 1988 a visiting postdoc, Riszard Andruskiewicz from Gdansk University, joined Silverman's laboratory and was asked to work on synthesis and characterization of 3-substituted GABA and glutamate analogues. He synthesized a set of 14 3-alkyl-GABA derivatives (Figure 1.4), 4-methyl GABA, and the two enantiomers, as well as seven glutamate derivatives. Most interestingly and also somewhat surprisingly, all of the GABA analogues were found to be activators of L-glutamic acid decarboxylase [24].

At that point (1989), they filed an invention disclosure and engaged in discussions with potential industrial partners, which led to start of collaborations with Upjohn Pharmaceuticals and Parke-Davis Pharmaceuticals. The most potent compound, *(R)*-3-methyl-GABA, did not display convincing anticonvulsant activity. Upjohn, concentrating on profiling the "best" compound, ended the cooperation at that point, while Parke-Davis scientists tested all derivatives and found that the isobutyl derivative resulted in very favorable pharmacological effects. This was somewhat surprising, as the activation of GAD was significantly weaker for this compound compared with the corresponding methyl derivative (*R*/*S*)-methyl-GABA (239 % activity of GAD at a concentration of 2.5 mM versus 143 % activation for the racemic isobutyl analogue) [24]. However, after synthesizing the two isobutyl enantiomers, they could confirm that (*S*)-3-isobutyl-GABA, later named pregabalin (Lyrica™), displayed one of the most pronounced anticonvulsant activities they ever tested.

Several years later, Parke-Davis scientists demonstrated that pregabalin binds to Ca^{2+}-channels, subsequently inducing calcium flux into the neuron. In turn this resulted in inhibition of glutamate and substance P secretion from excitatory neurons. So, in fact, the mechanism underlying the observed pharmacological effect of pregabalin, which was thought to be mediated by inhibition of GAD, was completely different. Inhibition of glutamate secretion does result in a similar pharmacological effect. Also the enhanced potency compared with other related derivatives could be explained by pregabalin being a substrate for the System L transporter, enabling active uptake into the brain [25]. Other compounds, like GABA itself, are not substrates of this transporter. Thus, their capability of crossing the blood–brain barrier is very limited.

Interestingly, in principle it only took the synthesis of 16 compounds to initiate the development of a successful drug candidate. Certainly many more compounds were produced and characterized in the Silverman laboratory, and still, the development of the actual drug required another 15 years until it was finally approved by the FDA in December 2004. But this drug development represents one of the rare cases where the final molecule was already obtained early on in the project. The originally assumed optimization rationale turned out to be not the correct one in various aspects, but by careful pharmacological examination, pregabalin was identified. This underlines the necessity to remain open to unexpected findings and keep the flexibility of adapting optimization goals and target values, or even the optimization strategy as a whole.

As a part of the deal with Silverman and the university, Pfizer (which had subsequently acquired both Park Davis and Upjohn), agreed to pay 4.5 % of global sales to the university, and Richard Silverman, who split his share with his coworker Andruszkiewicz, would receive 1.5 %. As Lyrica turned into a real blockbuster molecule, the university received an estimated US$ 1.4 billion in royalties.

On the topic of academic drug discovery, Silverman wrote in 2016: "*Academic scientists are not constrained by the requirement of making products to remain viable; therefore, shortcuts are not necessary, and tangential observations can be explored, which may lead to new discoveries. Because of this, academic invention needs to be encouraged in all areas of pursuit to allow new products to become available to society; industry should assist in financing the development of these products.*" [18]

1.4 Natural Product-Derived Drug Discovery

Another important contribution of academia to drug discovery is providing specific expert knowledge on particular research areas and techniques. This knowledge, acquired within the academic group of a professor throughout his complete academic career, may represent the long-sought solution to a specific problem that hampers progression of a compound to the market or prevents it from moving into clinical trials. This can be of particular value in the field of natural product research, as structural complexity is tremendous and compounds isolated from

plants, bacteria, or marine organisms represent a rich source of potential drugs. However specific skills, e.g. in isolation, structure elucidation, and synthesis, can be required to identify the active compounds and make them accessible for further exploration.

The screening of natural products for bioactivities led to a multitude of starting points for chemical optimization to clinical candidates or even directly to live-saving medications [26]. Several important examples discovered by academic groups are shown in Figure 1.5.

1.4.1 Antibiotics

Antibiotics figure prominently among the drugs discovered by academicians. Penicillin G represents one of the most influential findings in natural product research, saving the lives of millions of people. On 28 September 1928, Alexander Fleming, University of London, noted that on one of his bacterial culture dishes that was contaminated with a mold and that bacteria would die in proximity to the mold. He concluded that the mold produced an antibiotic substance. He published the results in 1929, [27] but the article and some following work did not receive much attention. The compound was difficult to isolate and it took until 1942 to reach the market [28]. Still today, penicillin G is on the WHO list of essential medicines, and in 1945, Fleming, together with Howard Florey and Ernst Boris Chain, received the Nobel Prize for medicine. Fleming also gave a beautiful description of his scientific finding, reminding us that chance is an essential part of scientific work – something scientists certainly cannot rely on, but should be prepared to spot and realize its potential.

> *One sometimes finds what one is not looking for. When I woke up just after dawn on September 28, 1928, I certainly didn't plan to revolutionize all medicine by discovering the world's first antibiotic, or bacteria killer. But I suppose that was exactly what I did* [29].

Streptomycin is another compound listed on the WHO list of essential medicines. It was isolated for the first time by Albert Schatz, a PhD student in the laboratory of Selman A. Waksman at Rutgers University in 1943. The results were published on 1 January 1944 [30], and the compound was quickly progressed to the clinics. Waksman, who also discovered several other important antibiotic natural products, among them actinomycin and neomycin, received the unshared Nobel Prize for medicine in 1952 "for his discovery of streptomycin, the first antibiotic effective against tuberculosis." However, it is highly debated, if the role of other contributors, in particular of Schatz, was downplayed [31].

Gramicidin S was discovered by Georgyi Frantsevitch Gause, a Russian microbiologist and his wife in 1942 [32]. By 1943 it was being used to treat wounded Soviet soldiers in World War II. Gramicidin S is produced by *Brevibacillus brevis* and consists of two identical fivemers, which are coupled to give a cyclic decapeptide.

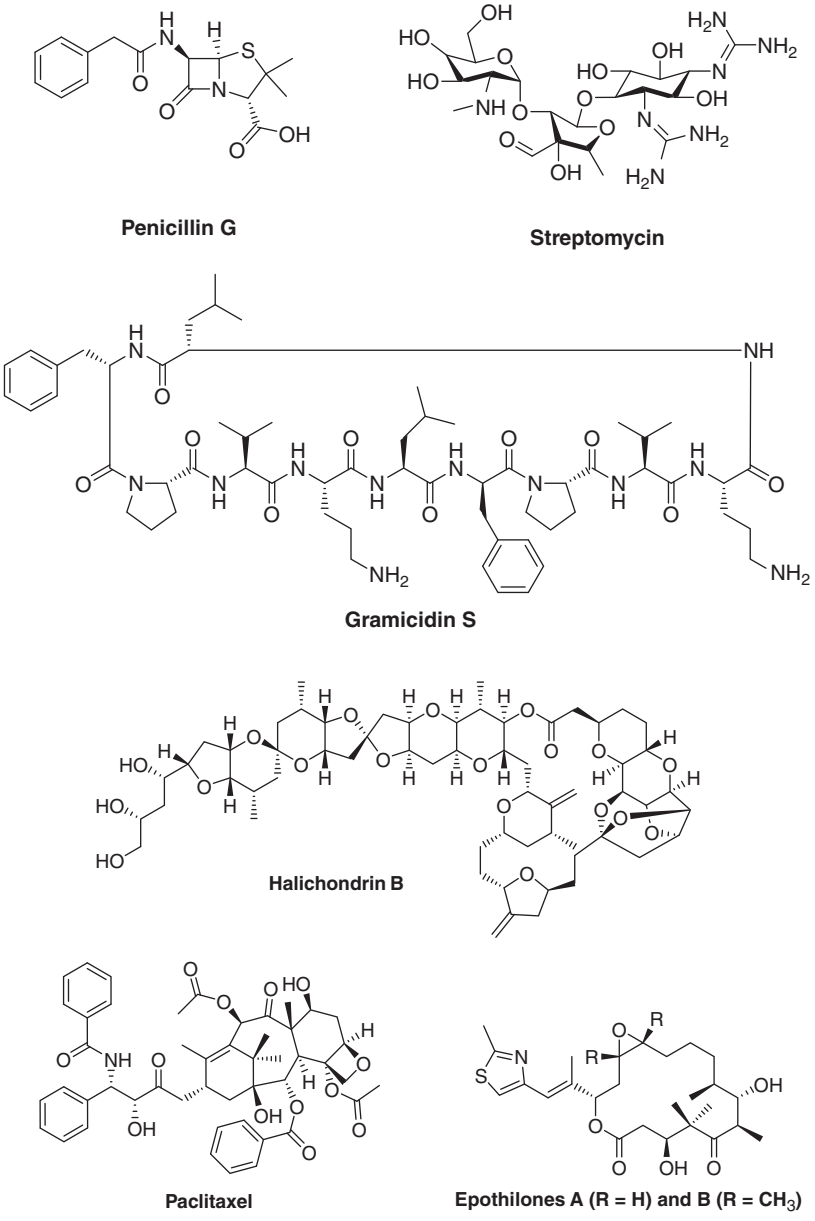


Figure 1.5 Lead structures isolated from natural sources.

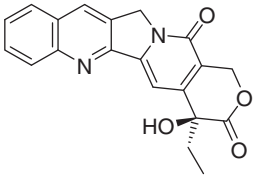
1.4.2 Anticancer Drugs

1.4.2.1 Camptothecin

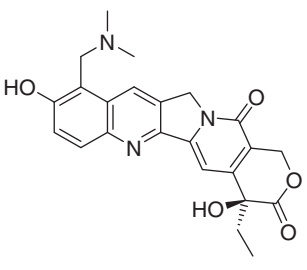
In 1952, the National Advisory Cancer Council discussed the promise of chemotherapy for curing cancer and came to the conclusion that the available knowledge was not sufficient to support establishment of a specific funding program for drug

discovery for cancer chemotherapy. However, in 1955, the Congress of the United States approved foundation of the Cancer Chemotherapy National Service Center (CCNSC) [33] and an associated budget of US$ 5 million for research on cancer. US$ 4.2 million were dedicated to grants supporting specific research proposals, while US$ 800 000 were reserved for acquisition and testing of new compounds. As a consequence, dedicated profiling laboratories were set up and a large compound collection was compiled. This effort was even strengthened in 1960, when the National Cancer Institute (NCI) partnered with the US Department of Agriculture (USDA) to collect plant and animal samples in search for natural products with potential anticancer activities. This alliance turned out to be very productive. Between 1960 and 1981, a total of 30 000 compounds was screened, and many pharmaceutically interesting structures were identified. At one of the involved profiling laboratories, the newly founded Research Triangle Park in North Carolina, chemists Monroe Elliot Wall and Mansukh C. Wani reported, among many others, the structure and activity of the natural product called camptothecin (Figure 1.6) [34].

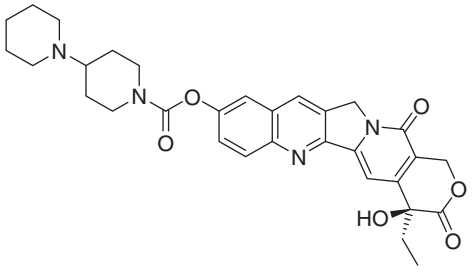
Camptothecin, isolated from bark and stem of the Chinese Happy Tree (*Camptotheca*), was first chemically derived through total synthesis by Stork and Schultz [35] (Cornell University) in 1971, quickly followed by syntheses by the Danishefsky [36] (University of Pittsburgh) and Winterfeldt (University of Hanover) laboratories [37]. Camptothecin was identified as an inhibitor of topoisomerase I, acting through binding to the covalent topoisomerase-DNA complex [38]. It is particularly toxic for cells in the S-phase of mitosis. Albeit camptothecin itself proved too toxic to be used as a chemotherapeutic agent in patients, it served as a valuable lead structure for the approved drugs topotecan (Hycamtin™, approved in 1996 for treatment of ovarian cancer, in 2006 for cervical cancer, and 2007 for treatment of small-cell lung



Camptothecin



Topotecan



Irinotecan

Figure 1.6 Camptothecin and approved derivatives.

carcinoma) and irinotecan (Camptosar™, a prodrug of topotecan approved in 1996 and used for treatment of colon cancer and small-cell lung cancer) (Figure 1.6). Both derivatives are derived through semisynthesis.

1.4.2.2 Taxol

The discovery of Taxol™ (paclitaxel, Figure 1.7) is another success story resulting from this campaign. In 1962, USDA botanist Arthur Barclay was on an excursion in Gifford Pinchot National Forest in Washington State to collect samples for the screening campaign. Among another 200 samples collected over the course of several months, he chose to take needles, twigs, and bark of the pacific yew. This turned out to be an important moment in cancer drug discovery.

Two years later, Wall and Wani at Research Triangle Park, North Carolina discovered a promising anti-leukemic and tumor inhibitory activity of an extract made from the collected stem bark [39]. However, the isolated yield from the dried bark was only 0.02 %. They contacted USDA and requested more material to supply further studies. In September 1964, Barclay went back to Gifford Pinchot National Park and collected another 30 lb of bark.

The yew tree itself has long been known to possess toxic properties. Almost any part of the tree is toxic but the red cup around the seeds is particularly hazardous. The lethal dose of needles of the common yew is estimated to be about 50 g for an adult. The toxic effects are caused by the contained taxine alkaloids (mainly taxine B), leading to cardiogenic shock [40]. These cardiac effects are distinct from the primary mechanism of action of Taxol and can be attributed to binding to ion channels. The main component of this activity seems to be taxine B (Figure 1.7). Its structure is related to that of Taxol, but besides other differences, it lacks the oxetane ring and

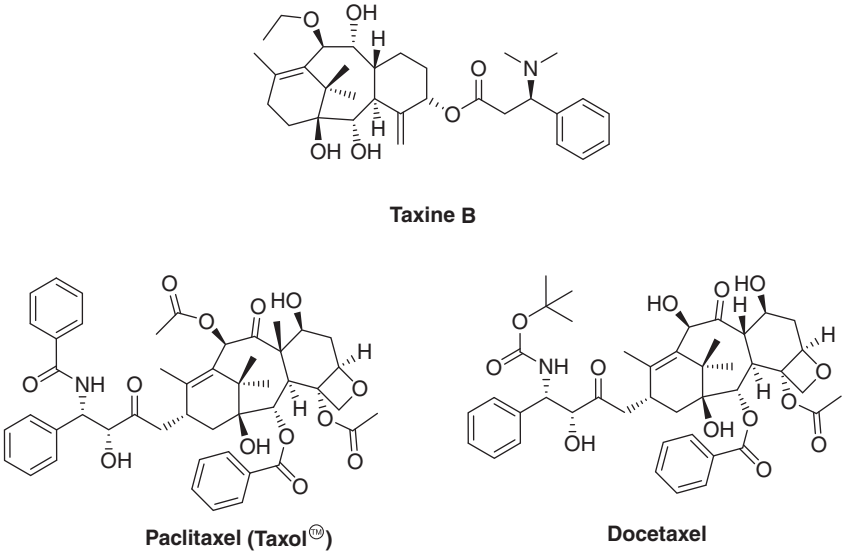


Figure 1.7 Taxol derivatives.

the benzoic amide and bears an *exo*-methylene group and a dimethyl amino residue. However, cardiotoxic side effects are also reported for paclitaxel.

Taxol did display interesting activities against various cell models of cancer and was moderately active in different models of leukemia. However, its solubility in aqueous media is very low. The initial overall interest in the compound was low, also as its availability was very limited. This changed quickly after new *in vivo* models were introduced at NCI in the early 1970s, and Taxol was found to be strongly active in a mouse model of melanoma. The pharmacological activity finally led to its nomination as a development candidate in 1977, triggering further examination.

In the same year, Susan Band Horwitz (Albert Einstein College of Medicine, Yeshiva University) was contacted by the NCI and was asked to explore the effects of Taxol [41]. She performed some initial experiments and observed that Taxol was capable of stopping replication of HeLa cells even at nanomolar concentrations due to its ability to induce mitotic arrest. Furthermore, she discovered a completely new phenotype. Cells treated with Taxol would be filled with stable microtubule bundles. In later research, it was determined that Taxol efficiently stabilizes microtubules, thus arresting cell cycle [42]. This new mechanism created a tremendous interest in Taxol. However, access to the compound was very limited. In fact, the bark of an estimated 3000 trees is needed to allow isolation of 1 kg of Taxol. Given that the tree will inevitably die after its bark is harvested and the pacific yew is a slow-growing species, the development process was slowed down significantly.

The intriguing complexity of the carbon backbone and its substitution pattern and the obvious need for alternative sources other than bark led to many academic groups pursuing synthetic approaches. Taxol's structure was elucidated by nuclear magnetic resonance (NMR) spectroscopy in 1971 by Wani [39], Holton [43], and Nicolaou [44] who reported the first two successful synthetic approaches to this challenging molecule, which may have marked a hallmark of natural product chemistry as this challenging molecule stimulated the whole field of natural product scientists. Other elegant syntheses were reported by Danishefsky [45], Wender [46], Kuwajima [47], Mukaiyama [48], and Takahashi [49], among others. However, the required complexity of the developed synthetic approaches limited their practical utility.

The first material for preclinical and clinical studies was still obtained from harvesting yew trees. Finally, in 1984 Taxol entered clinical phase 1 and phase 2 for ovarian cancer, which was initiated in 1985. Clinical profiling was delayed again by limited supply of the compound, but the first results were published by William McGuire (John Hopkins Center, New York) [50]. An initial response rate of 30 % was reported in women with cancer previously not responding to treatment. The increasing compound demands made further clinical profiling almost impossible. In addition, concerns about the environmental impact sparked public debate [51]. Specifically, it was discussed if it was appropriate to risk extinction of species to support clinical trials, which, if eventually successful, could potentially save some individuals. In 1987, NCI estimated that 60 000 lb of bark would have to be collected to support the requests for phase 2 studies, with another 60 000 lb required in 1989.

Previously, 6500 lb of bark had sufficed for supporting research for 10 years and only 2000 lb of bark were needed to provide the required amounts of Taxol from the

period 1962 to 1966. In 1989, 27 years after its discovery, no suitable route to access larger compounds quantities was within reach, and no patents protecting the compound were issued. The NCI decided to transfer the project to a pharmaceutical company for resolution of the remaining development issues and commercialization. At this time not too many companies were interested in cancer chemotherapy, as research costs were high and the expected chances of actually developing an effective drug were regarded as very small. Furthermore, in 1988 chemotherapy accounted for less than 3 % of the global drug market, compared with more than 17 % for cardiovascular drugs. Consequently, only four companies applied. The NCI finally decided to transfer rights to development under a cooperative research and development agreement to Bristol Meyers Squibb (BMS) in 1991. The contractual terms, which were granted to BMS, were very favorable; BMS received not only a market exclusivity for (the non-patented) Taxol but also an orphan drug status, the right to use all NCI-derived clinical data for applying for additional indications beyond ovarian cancer and, in a separate agreement with the Bureau of Land Management and the Forest Service, the right of first refusal on all products obtained from yew trees grown on public land [52]. This exclusivity spurred a public debate on granting a monopoly for plants on public land to a private enterprise and for giving exclusivity for a new cancer treatment based on data obtained by public funding. Also, concerns rose that yew trees could be harvested to the point of species extinction, as a result, the Pacific Yew Act was passed in 1992, which regulated yew harvesting to ensure careful management of remaining pacific yew resources and to provide sufficient supply of Taxol in the future. In 1992, BMS secured the name "Taxol" as a trademark – despite its utilization for more than 20 years – and created the new generic name "paclitaxel" for the drug.

The shortcomings of compound supply were finally resolved by combining results from different academic laboratories. Greene, Potier, and coworkers discovered that needles of the English Yew (*Taxus baccata*) contained large amounts (up to 0.1 %) of 10-deacetyl baccatin III. They developed a method to selectively silylate the hydroxyl group at C-7, followed by acetylation of the hydroxyl group at C-10 with enantiomerically pure results (Figure 1.8) [53]. Holton at Florida State University developed an effective β-lactam opening procedure. As he filed patent applications on this process, licensing by BMS, resulted in royalty payments of more than US$ 400 million to Florida State University.

Today, Taxol is a widely examined cancer treatment, with a total of 3875 studies on paclitaxel listed on clinicaltrials.gov on 1 March 2020. It is approved in the United States for the treatment of breast, pancreatic, ovarian, Kaposi's sarcoma, and non-small-cell lung cancers.

One limitation of Taxol is its very poor aqueous solubility of less than 0.01 mg/mL. The used formulation for clinical use as an intravenous injection is composed of a 1 : 1 mixture of cremophor EL (polyethoxylated castor oil) and ethanol, diluted with dextrose solutions or brine [54]. Cremophor, however, is not regarded as an ideal vehicle for human use, as it can create hypersensitivity, alter endothelial and cardiac muscle function and induce several other side effects. Furthermore, the concentration of cremophor that has to be used is unusually high.

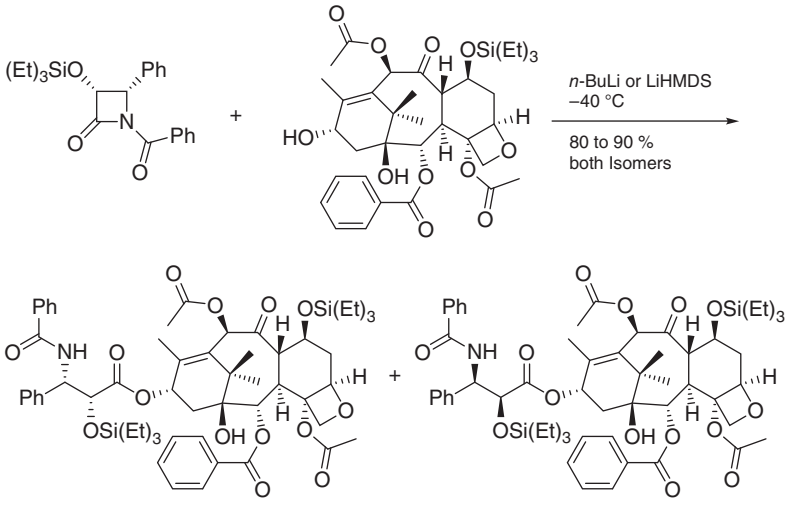


Figure 1.8 Semisynthetic approaches to Taxol. Source: Based on Denis et al. [53].

Neil Desai, a chemical engineer, and Patrick Soon-Shiong, surgeon and entrepreneur, met at a NCI organized conference on Taxol in 1992 and reasoned that it should be possible to derive a formulation, which was be better tolerated after application. After an intense optimization effort, they discovered that paclitaxel bound to albumin and formulated as nanoparticles can be a safer alternative which significantly improves the handling, solubility, and side effect profile of Taxol.

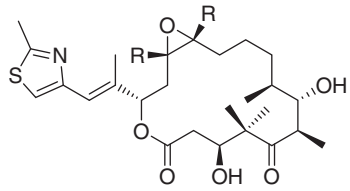
The compound, termed Abraxane™, could be dosed providing about 50 % higher paclitaxel amounts and still displayed better tolerability. Clinical studies reported improved response rates accompanied with improved tolerability [55]. This kind of innovation can be rather seen as an incremental one, but the specific approach can help utilizing the full potential of a given treatment. Abraxis, the company that was founded to drive the development of the reformulation platform and specifically Abraxane, was sold to Celgene in 2010 for US$ 2.9 billion.

Paclitaxel represents a perfect example for the impact of different contributions from individual researchers on the overall success of a drug. Here, isolation, structure elucidation, structure–activity relationship (SAR), access routes, and galenic aspects were tackled by a large number of scientists, contributing their specific experience and being able to make paclitaxel an important treatment option for various cancers.

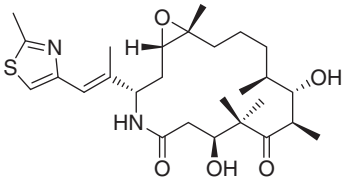
1.4.2.3 Epothilones

A related example is the work on epothilones (Figure 1.9). Initially isolated from the myxobacterium *Sorangium cellulosum* by Hofle et al. [56] in the German Federal Research Center Gesellschaft für Biotechnologische Forschung (GBF) in Braunschweig, the macrolides raised attention due to their structural and biological properties.

The formation of the 16-membered, highly functionalized ring system stimulated the creativity of many academic groups and spurred the development of new



Epothilones A (R = H) and B (R = CH_3)



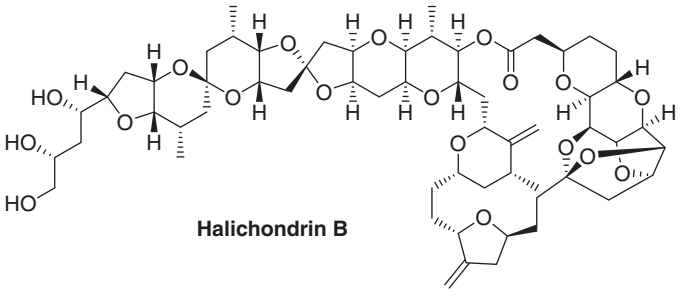
Ixabepilone

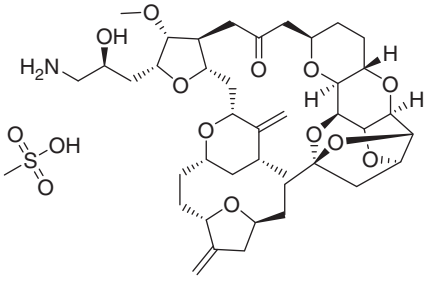
Figure 1.9 Epothilone derivatives.

and effective synthetic methods, e.g. ring closing metathesis for creation of the epothilone ring system. Among many others, the total syntheses reported by renowned academic experts such as Samuel Danishefsky [57], K.C. Nicolaou [58], Alois Fürstner [59], Dieter Schinzer [60], Eric Carreira [61], and Johann Mulzer [62] are particularly noteworthy, displaying a wide range of different approaches. Several companies, encouraged by synthetic accessibility of the core structures, got engaged in lead optimization programs. To date one derivative, ixabepilone (Figure 1.9), is used as a medication to treat advanced or metastatic breast cancer. It was developed by BMS [63] and received FDA approval in 2007.

1.4.2.4 Eribulin

While total synthesis was shown not to be a feasible production route for epothilone and Taxol derivatives, the approach still proved to be key for the development of another microtubule stabilizing agent. In 1986, Hirata and Uemura described the isolation of several family members of a novel class of natural products from the marine sponge *Halichondria okadai* [64]. This class, named halichondrins, consists of several family members that vary in their oxidation state. They show a remarkable structural complexity. Halichondrin B (Figure 1.10) possesses a staggering 32 stereocenters. In particular halichondrin B displayed outstanding cytotoxicity against a panel of 60 human cancer cell lines, which at that time was newly established at the NCI and became known as the NCI-60. Even more importantly, it showed excellent activity in *in vivo* cancer models. However, while it could be also detected in a few sponges of the *Axinella*, *Phakellia,* and *Lissodendoryx* families, its availability was extremely limited, as it could only be obtained in minimal quantities from the harvested sponges. Owing to the high potency of the compound, calculations indicated that only 10 g should be sufficient to supply clinical development and future need for commercialization was estimated to be between 1 and 5 kg. However, the producer organisms are rare, and it was calculated that at the time the available world supply of halichondrin B derived through extraction of one ton of harvested *Lissodendoryx n*. sp. 1 would amount to only 300 mg. *Lissodendoryx n*. sp. 1 is only found in an area of about 5 km^2 at a depth of 80 to 100 m, south of the coast of New Zealand. Calculations performed in 1993 estimated the total available biomass of *Lissodendoryx* to be only (289 ± 90) tons [65]. Yoshoito Kishi from Harvard University became interested in the unique structure of halichondrin B and set out to develop a synthetic access route. His main motivation was actually not in the anticancer properties of the drug,





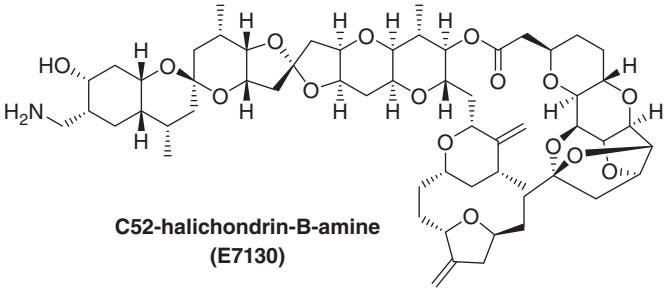


Figure 1.10 Structures of halichondrin B, eribulin mesylate, and E7130.

but at demonstrating the utility of the Nozaki–Hiyama–Kishi reaction in complex real-world examples. This was a grand challenge, but in 1992, Kishi and his coworkers succeeded in completing the first synthesis, which comprised a total of 128 steps [66]. Also in 1992, the NCI nominated halichondrin B for preclinical testing. Eisai decided to license the synthesis of halichondrin B patented by the Kishi laboratory and initiated a very unique and fruitful collaboration in which researchers at Eisai were supplied with advanced intermediates by the Kishi laboratory. This joint effort led to establishment of several analogues and the understanding of the scaffold's SAR. In the course of this exploration, the anticancer activity of halichondrin B could be associated with the right-hand side of the molecule, allowing a significant simplification of the molecule and finally resulting in the identification of E7389,

later termed eribulin (Figure 1.10). The SAR studies and associated synthetic challenges have been reviewed in detail [67]. Compound availability by total synthesis was essential to start clinical work. Preclinical data for eribulin were more than convincing, but for internal reasons Eisai could not pursue the compound at the time, so it was decided to explore the compound's effects through a NCI-sponsored phase 1 clinical trial. The first results were positive, so Eisai decided to sponsor further trials [68]. The compound received FDA approval in November 2010, only eight months after submission of the application. Today it is available in 50 countries for treatment of advanced metastatic breast cancer. It is the first drug that has shown improvement of survival in women with heavily pretreated metastatic breast cancer.

Albeit structurally significantly simplified, eribulin still bears 19 stereogenic centers and represents a showcase for organic synthesis, enabling access to structural complexity. Thorough optimization by the Kishi group [69] and by the Eisai process development group [70] led to significant improvement of the synthesis. Eribulin is now accessible in a 62-step synthesis and still represents the most complicated technical synthesis of a marketed drug to date. This record may be in danger, though, as the Kishi group recently reported the synthesis of an even more complex development candidate, termed E7130 (Figure 1.10). While the initial synthesis took a total of 109 steps, they managed to improve the syntheses to "only" 92 – significantly higher yielding – steps and obtained remarkable 11 g of material [71]. E7130 is now undergoing clinical trials and may eventually become a successor to eribulin.

1.4.3 Artemisinin and Artemether

An illustrative alternative example for the application of expert knowledge to tackle a roadblocking problem is the flow synthesis of artemisinin (Figure 1.11), developed by Peter Seeberger and coworker [72]. The discovery of artemisinin is a thrilling story of its own, which has been reviewed several times [73]. It is an essential drug for treatment of malaria, a devastating disease with a significant disease burden. It is estimated that every year 300 million children are infected with malaria. As this trypanosomal disease is widespread in developing countries, the production cost is a significant point to consider. Furthermore, the short half-life of the drug requires relatively high doses for successful treatment. Artemisinin has a challenging molecular structure, characterized by a complex molecular framework. The complex sesquiterpene endoperoxide is deemed too complex to be accessible by total synthesis in required quantities.

The Seeberger group used their knowledge of flow chemistry to design and optimize a flow process for the structurally related drug artemether [74] (Figure 1.11), utilizing *in situ* generated singlet oxygen for generation of a peroxide, followed by Hock cleavage. While this flow chemistry process was not implemented in the ultimate production process, which involves photooxidation of activated dihydroartemisinic acid and is capable of delivering average quantities of 370 kg per batch [75], the methodology still has great potential for future applications. In particular, it could be demonstrated on an impressive example that facile photochemistry can be applied in process chemistry by rethinking the production

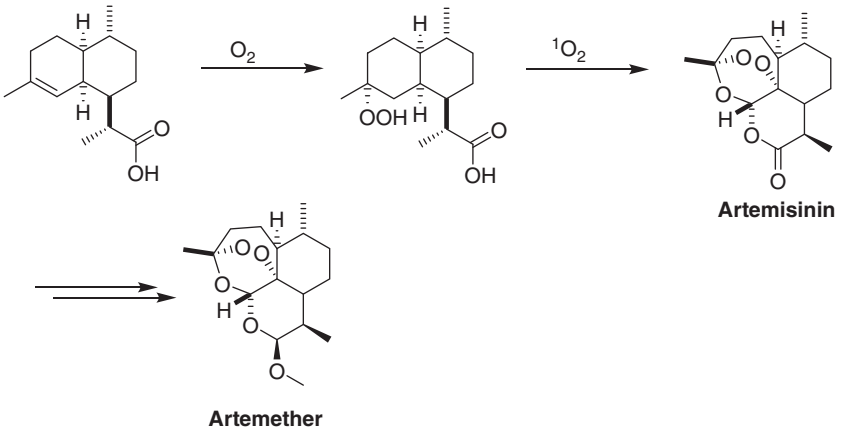


Figure 1.11 Seeberger's flow synthesis of artemisinin and artemether, essential drugs for treatment of malaria. Source: Based on Levesque and Seeberger [72].

process and converting the synthesis from large-scale bulk synthesis to steady small-scale synthesis by using a continuous process, which also is capable of delivering large quantities of drug substance.

1.4.4 Carfilzomib

The discovery of carfilzomib started from a regular literature search. Craig Crews (Yale University) was searching for new project ideas and reviewed past issues of the *Journal of Antibiotics*. He came across a compound called epoxomicin (Figure 1.12) [76] that caught his attention. The compound was isolated from an *Actinomycete* strain and displayed interesting cytotoxic activity against various cancer cell lines, as well as activity in an *in vivo* B16 leukemia model. The stereochemistry was not determined, but it had an exposed epoxide as a rather unusual structural feature. The molecule was actually discovered by Japanese researchers at BMS, but as the

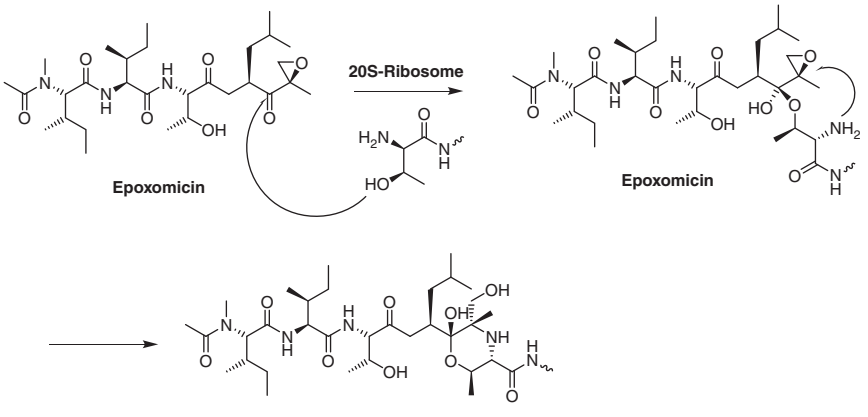


Figure 1.12 Epoxomicin binding to the 20S-ribosome. Source: Based on Hanada et al. [76].

mechanism of action was unknown and the drug-like properties of the compound were rather poor, BMS decided to drop the project. Crews did not intend to start a drug discovery project, but was rather interested in applying emerging chemical biology techniques to the molecule and unravel its mode of action. He completed the first total synthesis of the molecule, which also allowed determination of the previously unknown stereochemistry [77]. By employing a biotinylated derivative, he determined that epoxomicin specifically targets the proteasome [78]. The proteasome, a protein complex of about 1700 kDa, is responsible for degradation of misfolded proteins and exists in all eukaryotic cells and archaea, as well as in several prokaryotes. Shutting down the proteasome will significantly disturb normal cellular processes and will quickly kill the cell. However, cells with high replication rates should be more dependent on optimal functionality of the proteasome, thus opening an opportunity for cancer therapy. Several proteasome inhibitors, natural and synthetic, had already been reported in the literature [79], but their structures usually contained very reactive warheads, resulting in insufficient compound selectivity. Crews showed that epoxomicin, in contrast to other compounds with reactive warheads, selectively inhibited the proteasome. He started a collaboration with German Nobel laureate Robert Huber (Max Planck Institute for Biochemistry, in Martinsried, Germany) and solved the crystal structure of epoxomicin bound to the proteasome [80]. They discovered that epoxomicin reacted with the N-terminal threonine moiety of the 20S proteasome (Figure 1.12), forming a stable morpholino ring through successive epoxide opening by the terminal amino group and attack of the nucleophilic hydroxyl group on the ketone of epoxomicin. This specificity was remarkable, which prompted Crews and his coworkers to derivatize and improve epoxomicin.

After several rounds of optimization, first systematically varying the individual positions of the tetrapeptide and then combining the optimized residues in one molecule, they came up with a compound they later termed YU-101 (Figure 1.13)

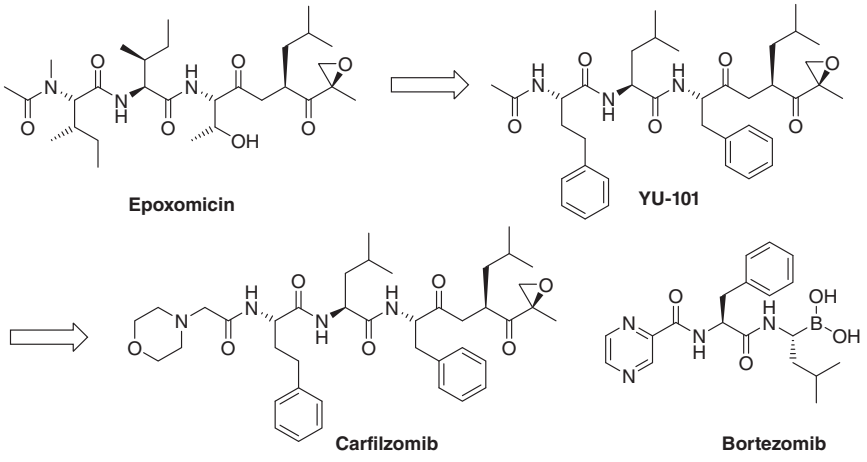


Figure 1.13 From epoxomicin to carfilzomib.

[81]. The compound did show significantly enhanced activity compared with epoxomicin and PS-341 (bortezomib), a dipeptidyl boronic acid derivative of epoxomicin developed by a biotech company called ProScript. Bortezomib was later acquired by Millennium Pharmaceuticals and became the FDA approved medication Velcade™, used for treatment for treatment of multiple myeloma and mantle cell lymphoma. YU-101 was licensed to Proteolix, a startup company founded by Craig Crews and Raymond J. Deshaies (California Institute of Technology). Proteolix was dedicated to the discovery of drugs targeting the proteasome. Scientists at Proteolix continued optimization and finally selected carfilzomib for preclinical and later clinical development. Proteolix was acquired by Onyx in 2009 for a nominal value of US$ 810 million. Carfilzomib (Kyprolis™, Figure 1.13) was approved by the FDA in July 2012 for treatment of advanced multiple myeloma and in 2015/2016 in combination with dexamethasone or lenalidomide and dexamethasone for treatment of refractory melanoma.

1.5 Biologic Drugs

1.5.1 Insulin

Contributions to drug discovery from academic groups are not limited to small molecules. Biologic drugs bear tremendous promise, and significant progress has been made to use them as therapeutics. The earliest reported example is the discovery of insulin [82]. The optimization of insulin to adapt short- and long-acting profiles has been described earlier in this series [83]. Interestingly, this work, albeit carried out in the laboratories of renowned pharmacologist John Macleod, was started by a rather inexperienced student, Frederick Grant Banting.

After returning from World War I, Banting worked as a lecturer in fall of 1920. Preparing for a lecture on the pancreas, a recently published article caught his attention describing the observation of surviving islets in an obstructed, atrophic pancreas. He assumed that degrading enzymes could be responsible for losing the active principle of pancreatic secrete and that these enzymes would likely be produced in acinar cells. Thus he developed the idea that by ligation of the pancreas and induction of atrophy, it may be possible to selectively destroy acinar cells and thus deplete degrading enzymes while maintaining the active blood sugar-lowering ingredient, which could then possibly be isolated by extraction. Enthusiastically he contacted Macleod, a proven authority in the field of diabetes. Macleod was skeptical that the approach could work, as many others had failed in isolating active pancreatic extracts before. He also easily noted that Banting only possessed textbook knowledge on diabetes, was not acquainted with recent literature on the topic, and also did not have the practical surgical experience to successfully perform the complicated procedures. However, after several meetings, Macleod agreed to offer him a (non-paid) opportunity to experiment in his laboratories and asked one of his student assistants, Charles H. Best, to assist Banting in the proposed research. They started their experimental work on 17 May 1921, but it quickly turned out that Banting overestimated his surgical skills and the dogs faded quickly. However,

Banting and Best subsequently formed an experienced team, and within 2.5 months they managed to treat a pancreatectomized dog with an extract isolated from excised pancreata of other dogs, which was able to transiently reduce its blood glucose. This result caused great excitement, but provision of extracts from duct-ligated pancreata was a laborious method with very limited throughput. In August 1921 they developed the idea to utilize fetal calf pancreata, being available from butchers, as these contained less acinar cells (which are responsible for excreting digestive enzymes and thus would lead to destruction of the – yet to be discovered – insulin). This turned out to be successful. The process was further improved significantly when they decided to use alcohol for extraction of the fetal pancreata. The alcohol extract was significantly easier to concentrate than the previously used saline solution. On 11 December 1921, they decided to use the established protocol on an adult bovine pancreas and for the first time, this extract also displayed a strong glucose-lowering effect. At that point in time, James Bertram Collip, a talented biochemist, was included in the team to produce the required extracts and particularly to optimize its production procedure. He thoroughly reworked the experimental procedures and discovered that the active principle of the extract was still soluble at high ethanol concentrations, which enabled precipitation of other proteins. At an ethanol concentration of 90 %, the active principle itself would precipitate, which enabled an effective purification protocol. Resuspension of the precipitate yielded the desired material. He also developed a more practical activity test, which relied on injecting an aliquot into a vein in a rabbit's ear, avoiding experimentation on pancreatectomized dogs. At the time, one unit of insulin was defined as "the amount of insulin required to reduce the concentration of blood glucose in a fasting rabbit weighing 2 kg to the convulsion level of 45 mg/dL (2.5 mmol/L)." Later, after the structure and molecular weight of insulin were determined, the earlier definition was replaced by one unit of insulin being defined as the "biological equivalent" of 34.7 μg pure crystalline insulin, still relating to the pharmacological effect of insulin on the initially used rabbits. The definition of a unit of insulin is still relating to these criteria, whatever the derivative or its molecular weight is considered.

The first type I diabetic patient was treated on 11 January 1922, less than eight months after the initial research was started. Injection of 7.5 mL of extract led to a marked, but temporary decrease of blood glucose and a significant reduction of excreted urinary glucose. No reduction of ketone bodies was noted. A sterile abscess developed at site of injection, likely resulting from remaining impurities of the extract. These results, albeit clearly far from optimal, spurred further research, and the next months were characterized by extensive production of material and further clinical testing. When treating the same patient on 23 January 1922 with a new extract carefully produced by Collip, a marked drop in glucose from 520 to 120 mg/dL was observed. Ketone bodies disappeared and the physical state of the patient improved significantly. Six more patients were treated in February and in March of that year an initial report on the clinical experiments was published [84].

The scientific success was clouded by a strong argument between the researchers. Banting felt early on that the more established and experienced Macleod would try to steal his original idea and claim the discovery as his own success. He believed

that Macleods' contributions were not significant and that his comments discouraged rather than encouraged Banting's research. He felt that the discovery of insulin was derived only through Best's and his own work. There was also some dispute about the value of the contributions of Collip, who, annoyed by the team atmosphere, announced that he would consider leaving the project and filing an individual patent on the purification procedure of insulin. It is reported that he and Banting even got into a physical fight over the project.

In the end, Banting and Macleod received the Nobel Prize for the discovery of insulin in 1923. Best and Collip were not included. The Prize was presented on 10 December 1923, less than 19 months after the group started their research. To this day, Banting remains the youngest Nobel laureate, being only 32 years of age when he received the Prize. Banting, upset with having to share the Prize with Macleod, initially wanted to reject the Prize but changed his mind later. He shared his monetary award with Best, as Macleod did with Collip. The decision of the Nobel committee also suffered criticism by other scientists, who had made important related discoveries before. In the case of insulin, particularly Georg Zuelzer [85], Ernest Scott [86], and Nicolas Paulescu [87] protested, but their contributions remained unacknowledged.

The discovery of insulin, albeit achieved many years ago, can still serve as a characteristic example of academic drug discovery. Clearly it began as an idea of an enthusiast, who was inspired by an ingenious thought and also clearly was not yet an expert in the research area he was about to enter. "Too much reading of the literature is inadvisable for wide diversity of opinion and confusion of thought" is a citation being connected to Banting. Also, the associated rivalry between the individual researchers is one point frequently observed particularly in academic settings. Necessarily, successful drug discovery is an interdisciplinary endeavor and calls for involvement of multiple experts willing to contribute their individual knowledge. Discussions on significance of individual contributions, e.g. reflected by debating first and last authorships, will poison the team spirit and easily compromise the joint research effort. It may even put the project as a whole at risk of a premature end. Also, decisions of the Nobel committee tend to cause criticism, particularly today, as the general research fields are broad and the selected questions are complex. Normally many scientists contributed valuable insights. With a maximal number of three laureates to be nominated for a particular topic, it is within the nature of this award that many scientists will find their contributions unconsidered.

1.5.2 Rituximab

In 1975, César Milstein, University of Cambridge, and his postdoctoral fellow Georges Jean Franz Köhler [88] first described the generation of monoclonal antibodies from hybridoma cells. Their high specificity and strong affinity quickly suggested that this concept may very well be suited for drug development. This discovery earned both of them the Nobel Prize for Medicine in 1984.

In 1980, a surface antigen of B-cells was described [89], which was isolated and further described and termed CD20 in 1988 [90]. CD20 is present on almost all

differentiation states of B-cells, except the immature ones, and found on cancerous as well as healthy B cells. A treatment targeting CD20 would accordingly eliminate all B-cells, but the immature ones, which then could form a new population after treatment, is finished.

Lee Nadler at Dana Farber Cancer Institute in Harvard University described and cloned the first antibody targeting a cancer-associated antigen called CD20. In a historic proof of principle study [91], he treated a first patient with this antibody. A transient response was observed, providing first evidence that targeting CD20 with monoclonal antibodies could be a viable therapeutic option to treat B-cell lymphomas.

Shortly after, the technology of generating chimeric antibodies was established, representing another milestone in the establishment of antibody therapies. Scientists at the University of Toronto and Columbia University demonstrated [92] that it was possible to generate antibodies bearing the human F_c region and bearing the mouse variable region. These antibodies were significantly less immunogenic, thus improving therapeutic prospects significantly.

Ronald Levy at Stanford University discovered that B-cell lymphomas were composed of monoclonal cell populations and, influenced by the work of Köhler and Milstein, he directed his research toward development of personalized monoclonal antibody therapy to target these lymphomas [93].

As early as 1982, a first patient was treated [94]. The promising results lead to formation of a start-up company called IDEC, which later turned into Biogen. The approach of developing personalized antibodies turned out too laborious and costly, instead CD20 was selected as a selective B-cell marker. As a direct result, rituximab [95], a chimeric monoclonal antibody targeted against CD20, was discovered. In the following clinical trial [96], tumor regression could be observed in about 50 % of the patients. This antibody represents a hallmark in the treatment of cancer and was the first antibody drug to be approved by the FDA for treatment of cancer in 1997. It was licensed to Roche and is used for treatment of various cancers like non-Hodgkin's lymphoma.

1.5.3 Alglucerase

Gaucher's disease is a rare disease that affects about 1 in 70 000 newborn children. It is characterized by a significant enlargement of the liver and spleen, fatigue, anemia, and decreased pulmonary function.

Dr. Roscoe Brady, a scientist at the National Institute of Health, was an expert on glycolipid metabolism. He discovered that symptoms of Gaucher's disease resulted from a deficiency of one specific enzyme, called α-glucocerebrosidase [97]. This deficiency results in a disability to process glucocerebroside, which causes accumulation of this sphingolipid in various organs and tissues. In following work, he proposed to isolate the enzyme and treat the disease by injecting the human enzyme directly into patients. Brady developed an isolation protocol from human placenta and could demonstrate a significant lowering of glucocerebroside levels in the liver after intravenous injection of the enzyme [98]. It is noteworthy that reoccurrence of glucocerebrosides in the blood of the patient was relatively slow.

However, it was quickly realized that this approach would not be a therapeutic option as, besides the short duration of the effect, isolated material from 40 placentae was required to treat 1 child with a single dose. Also, unfortunately, the isolation protocol could not be scaled up. After careful optimization of the isolation process, a facile deglycosylation strategy was developed to improve delivery of the glucocerebrosidase to macrophages, the location where a major fraction of the lipids was stored. Enriching the mannose content in the glycoside chains by treatment with exo-glycosidases resulted in a significantly more effective preparation [99].

In 1981, Sherdian Snyder, George M. Whitesides (Harvard University), and Henry Blair founded a company called Genzyme, dedicated to produce modified enzymes to provide them to the NIH for testing in clinical trials. Brady formed a relation with Blair, and Genzyme decided to start producing the enzyme required for clinical trials.

The first product, Ceredase™ (alglucerase), was still isolated from placentae, which required industrial scale purification and deglycosylation capacities. One year of treatment of a single patient required isolation of enzyme from 50 000 placentae. Finally, recombinant production of the enzyme, differing in only one position from placenta-derived protein could significantly simplify this process, also glycoengineering resulted in direct production of the optimized mannose-bearing oligosaccharide side chains. This product was named Imiglucerase (Cerezyme™).

Genzyme became a leading company in enzyme replacement therapy with the development of treatment options for lysosomal storage disorders being a cornerstone of its research. In 2011, Genzyme was acquired by Sanofi for US$ 20.1 billion. The detailed history of the development of enzyme replacement therapy for Gaucher's disease is described in more detail by Brady [100] and Deegan [101].

1.6 Conceptionally New Small Molecule Drugs

It is an important task of academic researchers to expand the lines of thinking of the scientific community and to open up new avenues for drug research, for instance, by demonstrating that new classes of enzymes can be drugged successfully or that novel mechanisms for modulating enzymatic activity can translate into *in vivo* efficacy, e.g. by addressing different binding modes, alternative pockets, or new mechanisms.

1.6.1 Histone Deacetylase Inhibitors

A first example is the discovery of vorinostat by Ron Breslow (Columbia University) and Paul Marks (Sloan Kettering). Its story has been told by Ron Breslow in the second volume of this series; he entitled the chapter "From DMSO to the Anticancer Compound SAHA, an Unusual Intellectual Pathway for Drug Design," which nicely sets the stage [102]. The journey started in 1971 from the observation by Virologist Dr. Charlotte Friend that a highly concentrated dimethyl sulfoxide (DMSO) solution can cause preferential differentiation of suspended murine erythroleukemia cells (MELCs) to red blood cells. This was certainly a stunning observation, but most industrial medicinal chemists would not have taken DMSO as a viable starting point for a drug optimization campaign. The connection between Paul Marks

and Ron Breslow occurred as a result of one of Marks' postdocs previously pursuing a fellowship in the Breslow laboratory. When options for starting a compound optimization program were discussed, she brought up the notion of getting in touch with him. Although Ron Breslow was a physical organic chemist by nature, he was immediately intrigued. The discussion started a collaboration that lasted for more than 30 years. Left with a thrilling phenotype, but no idea of a binding protein, nor any useful pharmacophore, optimization was challenging. They set out to test numerous small polar molecules and discovered that introduction of two polar side chains to DMSO led to a significant (~50-fold) increase in activity. Also, the team discovered that introduction of hydroxamic acids did improve potency. Recognizing the strong complexing properties of hydroxamic acids, they speculated that metal binding may play an important role. Further optimization led to suberoylanilide hydroxamic acid (SAHA). Structural similarity of SAHA to the natural product trichostatin A led to the assumption that SAHA could also target histone deacetylase (HDAC) [103]. This was confirmed in further studies [104], and zinc chelation turned out to be the key binding motif. SAHA is a pan HDAC inhibitor. Its moderate potency of 2.5 μM represents a good compromise of activity and avoiding toxic side effects (Figure 1.14).

In contrast to Silverman who established collaborations with large pharmaceutical companies directly, Breslow and Marks, together with Richard Rifkind (Memorial Sloan Kettering Cancer Center) and Victoria Richon in 2001, established a dedicated company to progress the compound. The newly founded Aton Pharma Inc. acquired the patent rights from Sloan Kettering and Columbia University. Through venture capital, a phase 1 clinical trial was performed [105], which demonstrated good tolerability and target engagement (histone acetylation in mononuclear cells and tumor tissue obtained through biopsies; pre- and post-treatment was analyzed). Also certain transient antitumor activities were observed in 37 cancer patients

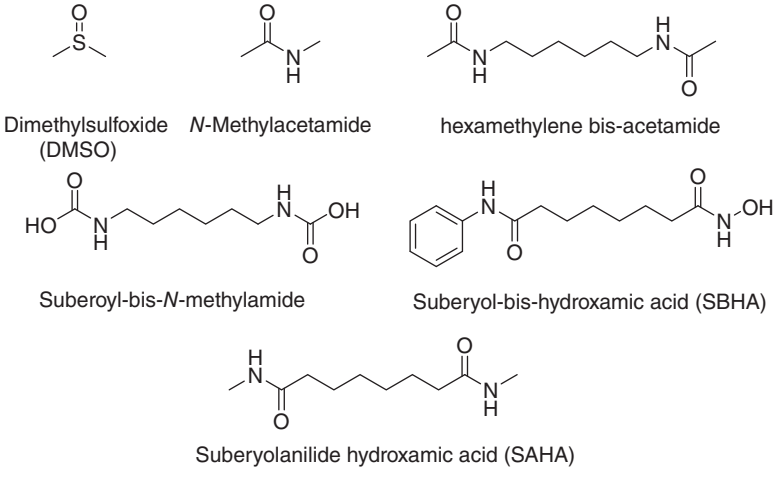


Figure 1.14 Structures of DMSO and hydroxamic acid derivatives leading to the discovery of vorinostat.

suffering either from solid tumors or hematological malignancies. In February 2004, Merck acquired Aton Pharma when SAHA was still in phase 2 clinical trials.

Vorinostat was approved in 2006 in the United States for the treatment of relapsed or refractory cutaneous T-cell lymphoma (CTCL), a rare form of lymphoma. Approval for CTCL was denied in Europe and trials for other indications have not been successful, yet the search for further indications, in particular in combination with other drugs, is still ongoing [106]. Even more importantly, vorinostat (Zolinza™) was the first approved HDAC inhibitor, opening the way to addressing a completely new class of therapeutically exploitable enzymes [107]. Five HDAC inhibitors have been approved by the FDA to date and several other drugs targeting that enzyme class are now in clinical development [108]. The development of the subtype selective HDAC inhibitor chidamide has been reviewed in Chapter 5, Volume 2 of this series [109]. The potential of epigenetics as demonstrated by HDAC inhibitors progressing into clinical development set the basis for a whole new research field and stimulated the formation of powerful public–private consortia like the Structural Genomix consortium. Started in 2004, to date this specific effort resulted in the publication of more than 2200 X-ray structures and 1700 scientific publications, and more than 75 chemical probes are available for biological studies on request, which tremendously increased the knowledge on the relevant protein families. This had significant impact on our understanding of structural requirements of epigenetic regulation, employing not only erasers like HDACs but also readers like bromo- and tudor-domains and writers like acetyl and methyl transferases.

1.6.2 Acyclic Nucleoside Phosphonates

Viral infections remain among the deadliest and most difficult to treat diseases. Influenza, herpes, smallpox, acquired immune deficiency syndrome (AIDS), and, more recently, COVID-19 developed into major health concerns for the global population. In the late 1950s, Yale scientist William Prusoff developed idoxuridine (Figure 1.13) [110] originally profiled for antibacterial and anticancer properties; it turned to be the first approved antiviral agent (1962). However, cardiac toxicity prevents its systemic application. This iodinated thymidine analogue can effectively inhibit the replication of various DNA viruses. In order to be activated, it needs to be converted to the corresponding triphosphate and can then be incorporated into viral, but also host cellular DNA. The modified DNA demonstrates higher susceptibility to erroneous transcription and strain breakage.

In 1976, Erik De Clercq (Rega Institute for Medical Research, Catholic University of Leuven, Belgium), a Belgian physician and virologist, and Antonín Holý, a Czech chemist (Czechoslowak Academy of Science, Prague) met at a symposium on synthetic nucleosides organized by the Max Planck Society and subsequently started a very successful collaboration [111]. At the time, Hóly was one of the most important chemists in Eastern Europe, De Clercq was an enthusiastic 35-year-old physician. In the course of this collaboration, they developed a deep and trusting relationship. Working on synthetic nucleoside analogues, they quickly

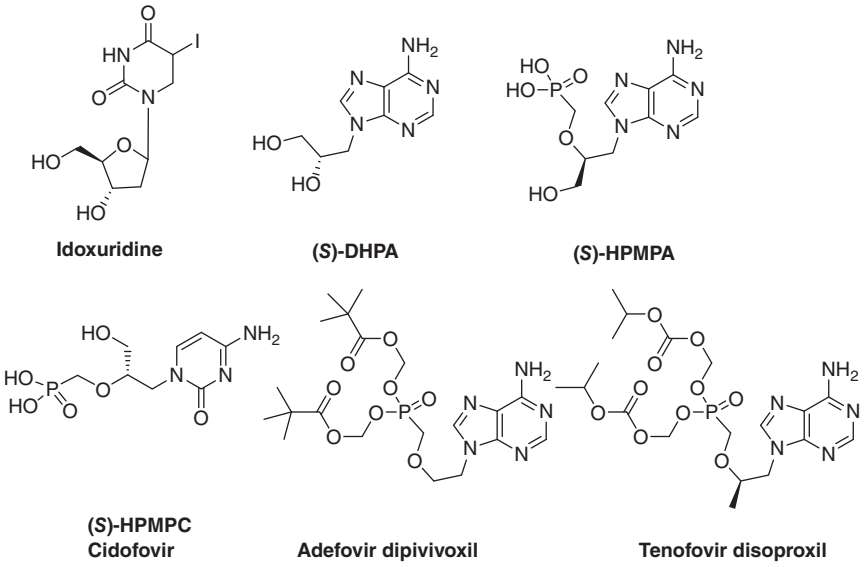


Figure 1.15 Structures of antiviral compounds developed by Hóly and De Clercq.

discovered (*S*)-9-(2,3-dihydroxypropyl)adenine ((*S*)-DHPA, Figure 1.15), the first acyclic nucleoside analogue [112] that displayed broad antiviral activity. Stimulated by reports on antiviral activities of simple phosphonates like phosphonoacetic acid, they set out to develop bioisosteric catabolically stable nucleotide analogues. Adding a phosphonate to the primary hydroxyl group led to the active monophosphate analogue, (*S*)-(3-hydroxy-2-phosphonylmethoxypropyl)adenine ((*S*)-HPMPA) (Figure 1.15) [113]. This compound displayed encouraging activity against various DNA viruses and seemed to work through a different mechanism than acyclovir, which had been described shortly before. They also discovered that the corresponding cytosine derivative ((*S*)-HPMPC, Figure 1.15) possessed an antiviral spectrum comparable to that of HPMPA [114]. This compound was later approved as cidofovir. Structural simplification of (*S*)-HPMPA, in particular removing the stereogenic center, led to adefovir, which was approved for treatment of hepatitis B in September 2002. Further optimization led to tenofovir, which, despite its chemical similarity, is more specific than adefovir (Figure 1.15) and does not inhibit herpesviridae. It is approved for treatment of human immunodeficiency virus (HIV), both alone and as combination therapy with emtricitabin, sold under the brand name Truvada™.

Tenofovir (Figure 1.15) is a prodrug that is quickly deprotected intracellularly, followed by double phosphorylation by adenosine monophosphate (AMP) kinase. The resulting triphosphate analogue cannot undergo complete dephosphorylation due to the presence of the phosphonate. Also, in contrast to other nucleotide drugs not bearing a phosphonate group (e.g. acyclovir), the activity of tenofovir does not rely on initial phosphorylation by viral kinases. It should thus possess a broad activity. It selectively inhibits the viral enzyme reverse transcriptase and displays favorable selectivity against human DNA polymerases. These compounds were

originally synthesized by Hóly in the form of their free phosphonates and displayed minimal bioavailability. Only when prodrug forms were developed [115] could the compounds be quickly absorbed after oral dosing.

After the original compounds within this class were discovered in the 1980s, preclinical exploration of several derivatives was performed at Bristol-Meyers. Upon merging with Squibb, the new company stopped projects involving these compounds, and the compound rights were returned to the inventing universities. Development of this compound class was reinitiated in 1989 by Gilead, leading to successful approval of three different new chemical entities (NCEs). In a license agreement, Gilead agreed to pay €11 million to the two universities, along with another 3% to 5% of net sales of different licensed products. Annual royalties to the Institute of Organic Chemistry and Biochemistry reached up to €90 million per year. Furthermore, in 2006 Gilead agreed to donate €1.1 million to create and sustain a Gilead Sciences Research Centre on campus. Hóly retired in 2011 and died on 16 July 16 2012, just two months after tenofovir was approved for treatment of HIV. On the very same day of Hóly's passing away, Truvada was also approved for prevention of HIV infections.

1.6.3 Darunavir

Arun Ghosh worked for several years with Merck & Co, where he acquired in-depth experience with the development of protease inhibitors. In 1994 he started his academic career in an independent laboratory at the University of Illinois-Chicago before moving to Purdue University in 2005. At the time that Ghosh entered academia, the HIV pandemic represented a global healthcare burden with no therapy available. The development of protease inhibitors was an active research area with great promise, but their use was associated with rapid development of resistance. Ghosh decided to tackle the problem of resistance development, pursuing a strictly structure-based approach starting from the X-ray structure of saquinavir bound to HIV protease. Saquinavir (Figure 1.16), developed by Roche, was the first inhibitor of HIV protease to obtain FDA approval in 1995. Its bioavailability is low and resistance occurs quickly with G48V being the key signature residue mutation of HIV-1 protease [116].

The approach pursued by the Ghosh group was to maximize interactions with the active site while simultaneously improving the overall compound properties, specifically the bioavailability. They assumed that the presence of multiple amide bonds could hamper compound absorption and tried to replace these by ether or sulfone groups. Furthermore, a thorough structural examination of multiple HIV mutants suggested that the protein backbone within the active site should superimpose very well for mutant proteases and only show minimal distortions, making this an optimal interaction point to maintain activity against these mutants [117].

Thorough compound optimization [118] led to the development of TMC-126 (Figure 1.16), which displayed impressive activity against the wild-type enzyme as well as against a wide range of mutants. Development of viral resistance against TMC-126 was delayed, and the resulting mutants were still sensitive to the vast

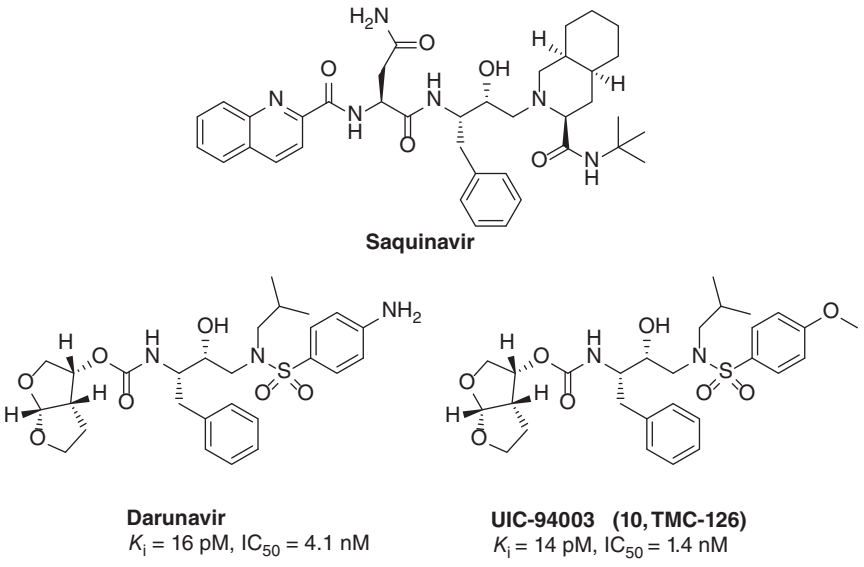


Figure 1.16 HIV protease inhibitors.

majority of other protease inhibitors, rendering the drug optimal for combination therapy.

Preclinical PK studies in rodents and dogs indicated low plasma levels of TMC-126. Further SAR studies led to the discovery of TMC-114, which later was termed darunavir in honor of its discoverer, Arun Ghosh [119]. In 1999, under the trade name Prezista™, darunavir was licensed to Tibotec Therapeutics, which was eventually acquired by Janssen Pharma. Darunavir was FDA approved in 2006. It is part of several combination products and is also listed on the World Health Organization's list of essential medicines. It displays exceptionally high binding potency ($K_D = 4.5 \times 10^{-12}$ M), which is 2 to 3 orders of magnitude higher than other HIV protease inhibitors [120].

1.6.4 Sunitinib

Inhibition of kinases, albeit omnipresent these days, is still a therapeutic principle known for less than 50 years. In 1986, Umezawa [121] reported erbstatin (Figure 1.17) as the first kinase inhibitor, targeting epidermal growth factor receptor. Because of high intracellular ATP concentrations, kinases were at that time widely believed to be undruggable. Achieving specificity seemed another impossible task considering the vast number of different kinases in the human body. However, in 1991, Joseph Schlessinger (Yale University) and Axel Ullrich (Max Planck Institute for Biochemistry in Martinsried) decided to start a company called Sugen, resulting from a collaboration of the two laboratories. The name Sugen is composed of the initials of the last names of the two founders, Schlessinger and Ullrich, combined with the suffix gen as an abbreviation for genetics. The company was

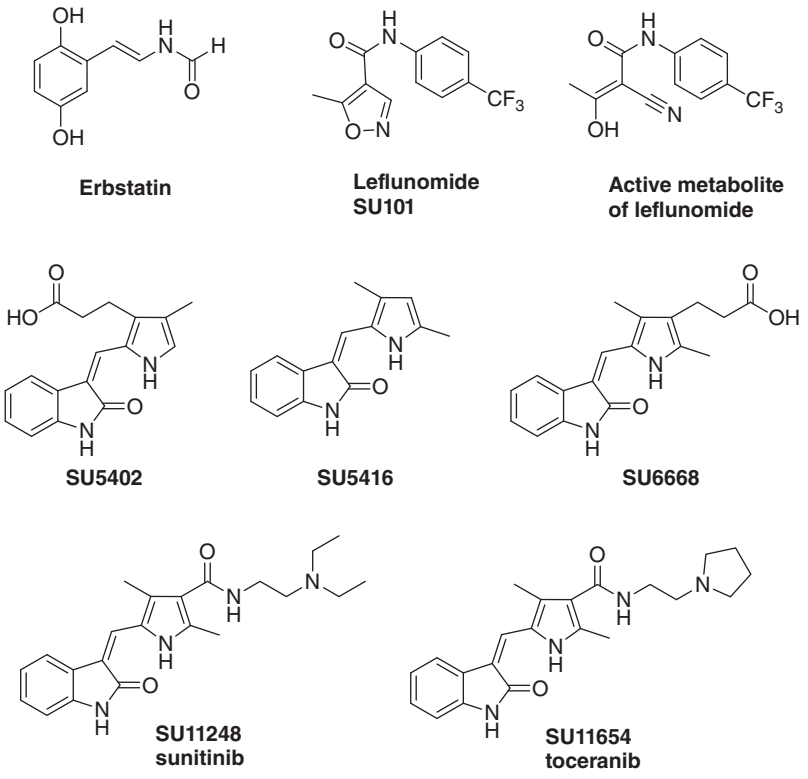


Figure 1.17 Kinase inhibitors developed by Sugen.

dedicated to developing anticancer drugs by manipulating intracellular signaling pathways and targeting kinases and phosphatases. In 1994 they filed the first IND for SU101 (Figure 1.17) targeting different cancer indications; however, it turned out that the structure of SU101 coincided that of leflunomide, which was under development by Hoechst Marion Roussell. The kinase inhibitory activity of SU101 was modest, in fact, the observed antiproliferative activity could later be linked to an active metabolite. Clinical development of SU101 failed and Sugen focused on another chemical series, the oxindoles. Cellular profiling and structure-based design using X-ray crystallographic data of SU5402 and SU6668 (Figure 1.17) in complex with the kinase domain of the Fibroblast Growth Factor (FGF) receptor [122] guided compound optimization. Sugen was acquired by Pharmacia & Upjohn in 1999 but continued research in a mainly autonomous manner. They progressed multiple compounds to clinical trials. One of them (SU11248, Figure 1.18) received FDA approval as sunitinib in 2006. Another compound, SU11654 (toceranib, Figure 1.18) received approval for treatment of canine tumors. Upon acquisition of Pharmacia by Pfizer in 2003, Sugen was closed. In testimony of Sugen's success, it is interesting to note that crizotinib, an anticancer drug marketed by Pfizer, also originated in the Sugen laboratories.

1.7 Sweet Spot for Academic Drug Discovery

The associated cost of late-stage clinical trials represents a major financial risk, even for large pharmaceutical companies. As a result, strong efforts are taken to increase the predictivity of development success and minimize risks associated with compound development. This leads to strict criteria applied to compounds before entering preclinical and clinical development and to a development process that is as standardized as possible. Certainly, minimizing risk may go along with minimizing chance. Often, difficult disease areas are abandoned, even if medical need is high. An example is the development of treatments for dementia, in particular Alzheimer's disease. The lack of suitable predictive models, combined with late-stage clinical failure of several approaches addressing amyloid and tau associated pathologies and a lack of other promising pathways, encouraged many companies to leave this area. Development of novel antibiotics is another area big pharma left some time ago. Here, the very limited successes of target-based screening campaigns and limited commercial prospects have turned into roadblocks. When powerful new antibiotics are identified, they are saved as last resort medications. This practice obviously contradicts the blockbuster model of big pharma, which is needed to finance the large headcount of the companies. In the case of antibiotics, the patents will likely expire before the drug sees broader use. Lastly, pharmacoeconomic considerations have led to big pharma leaving established disease areas like diabetes and cardiovascular diseases. Both diseases still represent major burdens to individuals and economy. The prevalence of diabetes is extremely high in Western countries (in 2018, 34.2 million Americans – or 10.5 % of the population – and 60 million people aged 25 years and over in Europe were suffering from diabetes), and even after more than 100 years of research, treatment still is only symptomatic. Cardiovascular diseases represent the most frequent cause of mortality [123] in Western countries. However, cost pressure by more restrictive reimbursement policies in many countries and availability of generic medications reduced the potential profit margin, rendering drug discovery in these disease areas less attractive.

These trends are even accelerated by company mergers, frequently resulting in erosion of the research infrastructure in the acquired company. Also, the number of different approaches addressing a specific target decreases since the number of companies pursing this target gets smaller. This leads to a significant reduction of available and clinically tested chemical matter, which will certainly have negative consequences on the availability of future medications.

The reduced internal research activity in several big pharma companies, however, opens up great opportunities for academic drug discovery. The motivation and primary goals of academic research are fundamentally different from industrial goals. In academia, research is mainly driven by scientific interest and curiosity. There is no need to create a business case and maximize potential revenue. To the contrary, medical need of neglected diseases or niche indications is an attractive field for research at universities. Novelty, scientific innovation, and increase of knowledge are key deliverables for academic researchers, and individual laboratories strive to prove their creativity and scientific aptitude. As a consequence, standard approaches

following predefined routine protocols are disfavored, as they are regarded as scientifically less valuable. Unusual and challenging projects are encouraged. Driver of projects are the individual interests of the respective principal investigators (PIs), who have frequently been working a long time in a specific area and thus have developed strong expertise on the one side but may also be able to more easily spot room for opportunity and innovation. Also, funding criteria, as defined by the NIH and other research organizations, include innovation as one of the five key factors for grant evaluation [124], making it harder to obtain public funding when pursuing approaches of incremental improvement. Time is not a key criterion, while industrial approaches follow strictly defined timelines that are required to ensure resource and budget planning, academic scientists can pursue their projects as long as they individually feel it is advisable and their curiosity and excitement persists. Of course, financial constraints apply to academics as well, as their budget normally is far more limited compared to industrial scientists, but as long as funding can be secured, consumables can be purchased, and a talented and motivated student is available, new results can be obtained. Shortfall of resources and high rejection rates at funding agencies have significant impact on prolonged discovery and development times, but as the PI can select the followed research interests autonomously, the projects may be paused but not halted. The academic research environment frequently results in long-term project relations. For example, the cooperation of Ron Breslow and Marks or Erik De Clercq and Antonin Móly lasted for more than 30 years.

A key success factor for drug discovery is smoothly connected interdisciplinary research. In academia, a large number of potential collaboration partners with complimentary experience are available. Hurdles for setting up a collaboration are low and frequently occur by meeting at scientific conferences, recommendation of colleagues or by spotting interesting publications and reaching out to the authors. In contrast to companies, where teams are assigned based on expertise, available resources, and budget constraints, in academic settings it is frequently observed that long-term relationships and friendship result in projects. And after all, if a collaboration does not work out, it is relatively easy to end it and search for a new cooperation partner. However, perhaps the most important point to consider is the vast number of academic research groups. Every group thrives for an individual, recognizable profile. Thus, a tremendous variety of projects and approaches is pursued. The individual activities of the research groups are quickly disseminated to the public, and these results can then stimulate other researchers working in related fields. This leads to rapid increase of knowledge and quick progress.

However, academia can also be a difficult place for drug discovery. Successful drug discovery requires access to a multitude of disciplines, specifically medicinal chemistry, *in vitro* and *in vivo* pharmacology, structural biology, and ADME to name a few. Many academic centers will not be able to provide all of this infrastructure. Furthermore, albeit timelines may be less pressing, access to resources can be very limited. Also teaching obligations, peer reviewing and grant writing consume significant amounts of time of academicians. This may lead to a lack of focus and slow down drug discovery projects or even prevent them from being successful. Also the focus on high-impact publications and the urge to publish results quickly in order

to secure further funding may be detrimental to patenting efforts and to securing intellectual property rights during the – sometimes long – optimization phase. It can be difficult for PhD students to get engaged in such kind of optimization campaigns when their future career may depend on successful publication outcome of their thesis. Even for the PI it may be challenging to maintain resources and patience with an optimization program when the first high impact publication is out and the tedious fine-tuning begins to optimize and eventually identify a preclinical candidate. The needed persistence requires access to a suitable infrastructure and required critical resources.

The in-depth knowledge on how molecules are successfully optimized is not regularly taught at many universities. This expertise resides mainly in industrial research units. Lack of medicinal chemistry experience and key optimization parameters frequently leads to optimization campaigns solely focusing on inhibition potency and neglecting other important parameters like metabolic stability, permeability, or solubility.

These campaigns frequently deliver mediocre hits with micromolar potency and insufficient physicochemical properties, selectivity or ADME parameters. When pursuing licensing negotiations with business development units at universities and pharmaceutical ventures, different opinions on the maturity and valuation of the project may lead to significant disappointment on both sides. While the university side may be convinced that the identified micromolar asset is just ready to go into clinical development, the pharma side may consider the obtained structure as an advanced hit or early lead, at best. This will undoubtedly complicate definition of milestones and payment terms. Here both sides have to openly interact and educate each other.

However, the previously mentioned examples demonstrate the invaluable contributions of academic medicinal chemists to drug discovery. Many new approaches have already been brought to practice. A large number of academically developed drugs is listed in the WHO list of essential drugs. Also financially, it can pay off for a university to pursue drug discovery and try to convert ideas and concepts from fundamental science into clinical practice. The reduced internal research in big pharma calls for new models, and more scientists with experience in the pharmaceutical industry are starting groups in academic settings and importing the knowledge of the drug industry into universities. Specifically, the increased demand for translational research calls for professionalized drug research at academic centers and will make drug discovery a vital and indispensable discipline at academic institutions.

List of Abbreviations

ADME	absorption, distribution, metabolism, and excretion
AIDS	acquired immune deficiency syndrome
AMP	adenosine monophosphate
BMS	Bristol Meyers Squibb
Ca	calcium
CCNSC	Cancer Chemotherapy National Service Center

CD20	cluster of differentiation 20
COVID-19	Coronavirus disease 2019
CTCL	cutaneous T-cell lymphoma
dL	deciliter
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
FDA	Food and Drug Administration
FGF	Fibroblast Growth Factor
g	gram
GABA	γ-aminobutyric acid
GAD	L-glutamic acid decarboxylase
GABA-AT	γ-aminobutyric acid aminotransferase
GBF	Gesellschaft für Biotechnologische Forschung
HDAC	histone deacetylase
HIV	human immunodeficiency virus
*i*Bu	isobutyl
IC_{50}	half maximal inhibitory concentration
*i*Pr	isopropyl
kg	kilogram
K_i	dissociation constant of an inhibitor
L	liter
MELC	murine erythroleukemia cells
mg	milligram
mL	milliliter
mmol	millimol
NCE	new chemical entities
NCI	National Cancer Institute
N-Lost	nitrogen lost
NMR	nuclear magnetic resonance
*n*Pr	*n*-Propyl
RA	rheumatoid arthritis
rac	racemic
SAHA	suberoylanilide hydroxamic acid
SAR	structure–activity relationship
*s*Bu	*sec*-butyl
S-lost	sulfur-lost
USDA	US Department of Agriculture
WHO	World Health Organization
µg	microgram
µM	micromolar

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Biography

Oliver Plettenburg studied chemistry at the University of Wuppertal and obtained his PhD under the supervision of Prof. Hans-Josef Altenbach in 2000. He then moved to The Scripps Research Institute, La Jolla, to work under the guidance of Prof. Chi-Huey Wong, where he focused on the synthesis of glycolipids. After this he joined the pharmaceutical industry at Aventis Pharma, working first in classical medicinal chemistry and developing projects from hit and lead optimization phase all the way to early clinical trials. Later he was more and more involved in establishing alternative concepts in pharmaceutical research, and lastly he held positions as "Head of Chemical Biology" and "Head of Biosensors and Chemical Probes" at Sanofi-Aventis, Frankfurt, Germany. From 2016, Dr. Plettenburg serves as founding director of the Institute of Medicinal Chemistry of the Helmholtz Center Munich, and he is also a full professor for Medicinal Chemistry at Leibniz Universität Hannover. His research focuses on hit and lead optimization to open up new treatment options for severe diseases like diabetes, lung, inflammatory, or infectious diseases. Furthermore, he develops innovative targeted and smart drug delivery methods and works on the synthesis of novel imaging agents for *in vivo* monitoring of pathogenesis.